REMARKS

Claims 1-6, 8-14, 17, 19, 21-46 and 83-93 are pending and under examination.

Rejections Under 35 U.S.C. § 103(a)

Nucleic Acid Claims

Claims 1-6 and 8-13 remain rejected, and claims 90 and 91 are newly rejected, under 35 U.S.C. § 103(a) as allegedly obvious over Severino et al., *Cell*, 1989, 57(l):ll-19 (Severino), in view of Stoller et al., *J. Cell Biol.*, 1989, 108: 1647-55 (Stoller), U.S. Patent No. 5,118,666 (Habener), U.S. Patent No. U.S. 5,891,671 (Suzuki), and Patel et al., *CIBA Foundation Symposium*, 1995, 190: 26-50 (Patel).

Applicants respectfully traverse this rejection because the Office has failed to establish a *prima facie* case of obviousness against the claims. It does not identify a suggestion or motivation that would have led a person of skill in the art, at the time of the invention, to combine the teachings of the five cited references. The rejection also fails to demonstrate that skilled practitioners would have had a reasonable expectation of success, based on the five references, to arrive at the specific nucleic acid constructs recited in the claims.

1. Motivation or Suggestion to Combine

Applicants respectfully submit that the Office has pointed to no proper motivation or suggestion to combine Severino, Stoller, Habener, Suzuki and Patel to arrive at the presently claimed nucleic acid constructs and cells. As alleged evidence of a motivation to combine, the Office Action provides the following characterization of prior art (at pages 3-4 of the Office Action paper):

> With respect to motivation, besides the therapeutic reasons for making GLP-1 taught by Habener, Suzuki teaches that it is desirable in the art to utilize the expression of a chimeric protein for a number of peptide production including GLP-1 (column 5, lines 13-22), that enzymatic cleavage such as furin can be used for separating a target peptide (column 1, lines 15-18), and that it is expected that the peptide hormone is not damaged and the processing enzyme is applicable to a

> wide variety of peptides, therefore, the development of such production methods has been desired in the art (column 1, lines 36-49), and Sevarino and Stoller teach that the pro-region of preprosomatostatin can be used for targeting a heterologous peptide to regulated secretory pathway. The teachings of Suzuki, Sevarino and Stoller provide strong motivation to make the construct as claimed because it would allow the target peptide to be secreted, which would greatly facilitate the purification process.

Applicants respectfully disagree with the above-quoted assertion, and in particular to the assertion that Suzuki, Sevarino and Stoller provide a "strong motivation" to make the presently claimed constructs and cells. None of the references, singly or in combination, would have provided skilled practitioners with any motivation to arrive at applicants' constructs and cells.

Habener discloses recombinant GLP-1, but it does not teach, suggest, or provide any guidance whatsoever for linking GLP-1 to any peptide domains, much less a signal sequence or a somatostatin pro-region. Suzuki teaches GLP-1 chimeras for expression as insoluble bodies within *E. coli*. Consistent with this production method, i.e., non-secreted insoluble production, Suzuki discloses no teaching or suggestion that would motivate a skilled practitioner to (i) link GLP-1 to a eukaryotic signal sequence and somatostatin pro-region or (ii) combine Suzuki with a reference about other peptide production methods. Sevarino teaches a rat somatostatin prepro-region linked to an anglerfish somatostatin. Sevarino does not teach or suggest linking a somatostatin prepro-region to a non-somatostatin peptide, e.g., the GLP-1 taught by Habener and Suzuki. Stoller teaches a large peptide, α-globin, linked to an anglerfish somatostatin prepro-region. Thus Stoller contains no teaching or suggestion to link a somatostatin prepro-region to a small, non-somatostatin peptide, as taught by Habener, and Suzuki. Applicants respectfully submit that the Office has failed to point to a motivation found in these references that would have led skilled practitioners to combine the teachings of the cited references and arrive at the presently claimed constructs. These are isolated references with absolutely no suggestion of combination. The suggestion to combine comes entirely and only with hindsight guided by Applicants' disclosure. Each of the cited references is more fully discussed below.

Habener discloses GLP-1 peptides and a general suggestion to make GLP-1 peptides for their therapeutic value. However, Habener, provides no indication that GLP-1 should be linked

to any other peptide domain, much less the currently recited signal peptide and somatostatin pro-region domains. The disclosed therapeutic value of GLP-1 provides no motivation for linking GLP-1 to the currently recited peptide domains.

Suzuki describes a general method that includes overproducing chimeric proteins (including GLP-1 chimeras) that accumulate in *E. coli* cells as insoluble inclusion bodies, which are subsequently solubilized under conditions that allow target peptides to be cleaved from their chimeric precursor. Column 2, lines 6-11. Thus, Suzuki provides no suggestion at all for altering the disclosed constructs to achieve secretion. In fact, the teaching is to recover the expressed chimeric protein as an **insoluble cellular** component. Furthermore, Applicants respectfully point out that (i) signal peptide and somatostatin pro-region domains exert their biological functions in the secretory system of mammalian cells, and (ii) *E. coli* lack the defining components (e.g., the endoplasmic reticulum, Golgi apparatus, etc.) of the mammalian secretory system. Therefore, Suzuki does not even hint at a reason for linking a signal peptide and somatostatin pro-region to a target peptide (e.g., GLP-1). For these reasons, skilled practitioners would not have been motivated to combine Suzuki with Sevarino, Stoller, or any other art, in an attempt to arrive at the presently claimed constructs.

Sevarino, which teaches the use of rat prepro-region to correct the targeting deficiencies of anglerfish somatostatin in mammalian cell lines (see, e.g., page 11, last sentence of Summary) adds nothing to the disclosures of Habener and Suzuki. The "small heterologous peptide" alleged by the Office Action to be taught by Sevarino is a somatostatin. It is clear that Sevarino's focus was primarily on investigating the role of prepro-regions in intracellular sorting of somatostatin precursors (see, e.g., page 17, last sentence of Discussion). Sevarino contains no teaching or suggestion that somatostatin prepro-regions would be generally useful for the expression of small peptides **other** than somatostatin. Therefore, a skilled practitioner would not have been motivated to combine the teachings of Sevarino with art relating to the production of non-somatostatin peptides.

Stoller discloses an anglerfish pro-region linked to chimpanzee α-globin (referred to by Stoller as "PRO-GLO"). Stoller, like Sevarino, also adds nothing to the disclosures of Habener

and Suzuki. Stoller's PRO-GLO chimera does not comprise a small, non-somatostatin peptide. Nowhere does Stoller teach, or even suggest, that a somatostatin prepro-region should, or even that it could, be used for secreting small non-somatostatin peptides. Therefore, a skilled practitioner would not have been motivated to combine Stoller with Habener or Suzuki, in such a way as to arrive at the presently claimed constructs.

Although the Office Action has not discussed Patel in further detail in the present rejection, applicants reiterate here their position that Patel discloses that mammalian pro-protein convertases such as furin, PACE4, and PC1-6, mediate cleavage of prosomatostatin. Patel does not teach or suggest constructs for expressing of small, non-somatostatin peptides. Therefore, Patel does not teach, or even suggest, the currently claimed nucleic acid constructs.

Applicants respectfully submit that skilled practitioners would not have been motivated by Habener, Suzuki, Sevarino, Stoller and Patel, singly or in combination, to combine the teachings in these references to arrive at the presently claimed invention. Applicants disagree in particular with the Office's assertion that Suzuki, Sevarino, and Stoller provide a "strong motivation to make the currently claimed nucleic acid constructs" (see the Office Action at page 4). The Office has not explained why a skilled artisan would have been motivated to combine Sevarino and Stoller, which relate to the prepro-region of somatostatin precursor and its role in directing transport through the regulated secretory pathway of endocrine cells, with Suzuki, which relates to cellular non-secreted overexpression of chimeric proteins in *E. coli* (which, as noted above, lack the secretory machinery of endocrine cells) in insoluble form. The only alleged motivation provided by the Office can be found in the broad, conclusory statement that such a combination "would allow the target peptide to be secreted, which would greatly facilitate the purification process." (Office Action at page 4). However, the Office has not pointed to evidence that (i) skilled practitioners needed, or even wanted, to simplify the process disclosed in Suzuki or (ii) that the claimed constructs would actually simplify it. Furthermore, even if the art expressed a general desire to facilitate purification, such a desire would not have rendered Applicants' constructs or cells obvious. The goal of simplifying purification simply does not, on its own, suggest the specific constructs for achieving that goal. Applicants maintain

their position that the Office has provided no evidence of any motivation, much less a "strong motivation," to combine these references to arrive at the presently claimed invention.

2. Reasonable Expectation of Success

Applicants also submit that no skilled practitioner would reasonably have expected to be successful in making and using the presently claimed nucleic acid constructs, in view of the cited references, either singly or in combination. In particular, Applicants disagree with the Office's assertion (at page 4):

> The combined results by Severino and Stoller strongly indicate that the expression system comprising prepro-region of a presomatostatin can be suitable for a broad range of peptides with different sizes.

The combined results of Severino and Stoller, however, amount to only two prepro-region containing chimeras, neither of which includes a small, non-somatostatin peptide. One (Severino) does not really include a heterologous peptide, rather it includes a homologous somatostatin. The other (Stoller's) includes a large heterologous peptide. The disclosure of only two chimeras, neither of which includes a small non-somatostatin peptide, would not have created in a skilled practitioner a reasonable expectation that a construct encoding a signal peptide, a pro-region of a somatostatin, and a small peptide other than somatostatin pro-region, could successfully be made and used for expressing small non-somatostatin peptides.

Examination of Severino reveals yet another reason why Severino would not have provided the requisite reasonable expectation of success. The Severino chimera merely replaces the somatostatin domain of rat prosomatostatin with a structurally and functionally homologous anglerfish somatostatin domain. Applicants point out that the successful swapping of homologous somatostatin domains simply would not have suggested to skilled practitioners that non-homologous (e.g., non-somatostatin) small peptides could successfully be used in place of the rat somatostatin domain disclosed in Severino. Thus, Severino (alone or in combination with Stoller), does not provide a reasonable expectation that the claimed constructs could be made and used to express small non-somatostatin peptides.

Stoller discloses a pro-region linked to a large α-globin peptide, which, of course, is not a small polypeptide as defined by the specification (see, e.g., page 52, line 24 to page 53, line 3). The use of a pro-region to express one large, heterologous peptide is not indicative that this prosomatostatin prepro-region is generally suitable for directing expression of a broad range of peptide sizes. Moreover, successful expression of a large peptide in any particular system does not fairly suggest that the same expression system can be used to direct the expression of small peptides in an active form.[1] Thus, Stoller (alone or in combination with Sevarino), would not have provided skilled practitioners with a reasonable expectation that a construct encoding a signal peptide and a pro-region of a somatostatin could successfully be made and used for expressing a small, non-somatostatin peptide.

Thus, the combined teachings of two chimeras in Sevarino and Stoller simply do not provide a reasonable expectation of success in expressing a broad range of pro-region linked peptides. Contrary to what is suggested in the Office Action (see the Office Action at page 4), Applicants have not requested that the Office point to evidence that would have provided practitioners with a complete certainty of success, only to evidence that would have provided practitioners with a reasonable expectation of success. Applicants respectfully submit that the Office has failed to provide such evidence.

Based on the above, applicants submit that the Office has not demonstrated (a) a suggestion or motivation and (b) a reasonable expectation of success that would have led a skilled artisan to combine the cited references in such a way as to arrive at the currently claimed nucleic acids. Absent these, the Office has not made a *prima facie* case of obviousness against the claims.

It appears that the Office has done no more than piece the invention together using applicant's specification as a template, which constitutes impermissible hindsight. Applicants respectfully submit that the Office appears to have used applicants' specification for at least the knowledge that the claimed nucleic acid constructs, which include a pro-region of a pro-

[1] As the current specification notes, small peptides are susceptible to degradation, they are not always efficiently synthesized, and in the case of small peptide hormones they must be properly processed in the cell. See p. 1, lines 7-15 and p. 53 lines 20-29.

somatostatin, would work for expressing small non-somatostatin peptides. The specification clearly teaches that among several pro-regions tested, only the somatostatin pro-region effectively directed expression of small non-somatostatin peptides. For example, the specification provides (at page 53, lines 21 to 24):

> [A] number of pro-regions from other precursors were used to replace the somatostatin pro-region and it was found that other pro-regions do not have the ability to direct biosynthesis and secretion of small peptides in this context.

As taught by the specification, not all pro-regions are useful to direct the biosynthesis and secretion of small peptides. The Office has pointed to nothing in the cited art that suggests why a skilled practitioner would have picked a pro-region of a pro-somatostatin, instead of other art-known pro-regions, to express small non-somatostatin peptides. Applicants respectfully submit that the information missing from the prior art appears to have been gleaned from applicants' specification. Therefore, applicants maintain their position that it appears that the Office has engaged in impermissible hindsight here.

For the reasons presented above, applicants respectfully request that the rejection of claims 1-6, 8-13, 90, and 91 be reconsidered and withdrawn.

Non-Endocrine Cell Claims

Claims 14, 17, 19, 21-35, 37-46, and 83-89 remain rejected, and claims 92 and 93 are newly rejected under 35 U.S.C. §103 (a) as allegedly obvious over Sevarino et al., *Cell*, 1989, 57(l):ll-19 (Sevarino), in view of Stoller et al., *J. Cell Biol.*, 1989, 108: 1647-55 (Stoller), U.S. Patent No. 5,118,666 (Habener), U.S. Patent No. U.S. 5,891,671 (Suzuki), and Patel et al., *CIBA Foundation Symposium*, 1995, 190: 26-50 (Patel) as applied to claims l-6 and 8-13 above, and further in view of Warren et al. Cell, 1984, 39:547-55(Warren), and U.S. Pat. No. 6,531,124 Bl (Selden). Applicants respectfully traverse this rejection for the reasons discussed below.

Applicants disagree with the rejection on several grounds. First, the rejected claims recite novel and non-obvious nucleic acids. The use of such non-obvious nucleic acids in non-endocrine cells is also (necessarily) not obvious. Arguments are presented above and in

applicants' previous Replies for the non-obviousness of the recited nucleic acids. Second, Applicants maintain that the Office has not supplied the required motivation and reasonable expectation of success for combining the seven references listed above to arrive at the rejected claims. This second reason for traversal is discussed below.

As an initial matter, Applicants respectfully submit that their previously submitted arguments are not impermissible attacks on the cited references individually, contrary to what is asserted in the Office Action at page 6. Applicants did not review the references in isolation. Rather, as part of the response, Applicants scrutinized each of the cited references individually for any suggestion that they should be combined with the other cited references. This is clearly an appropriate way of responding to an obviousness rejection. It is also worth noting that, in at least some cases, Applicants were responding to statements in the previous Office Action that unambiguously pointed to *individual* references as supplying alleged motivation to arrive at the claims reciting non-endocrine cells (see, e.g., the Office Action mailed September 9, 2003 at page 12). The present Office Action does so as well. For example, page 6 of the current Office Action states, emphasis added:

> "The *point to learn from Selden* is that small peptides such as GLP-1 can be expressed in either primary or secondary cells, which are useful for gene therapy, and *provides strong motivation for choosing these cells*."

In arguing against the rejection, it is clearly permissible for Applicants to point out the deficiencies in Selden that prevent Selden from supplying the motivation to make or use the currently recited cells. As applicants previously stated, "[t]he fact that Selden discloses expressing GLP-1 using a primary (presumably non-endocrine) cell transfected with a DNA encoding GLP-1 does not provide a suggestion or motivation to produce the claimed cell having the specific recited construct." See pages 14 and 15 of the Reply filed March 03, 2004. This statement respectfully reminds the Office that the successful operation of one expression system in a cell line does not indicate a reasonable expectation for the success of an unrelated expression system. Thus, the operation of Selden's expression system (which is completely unrelated to the currently recited constructs) in non-endocrine cells provides no motivation for using the currently recited nucleic acid constructs in the same cells.

The Office Action also points (at page 6) to Warren and Patel's results "of using the somatostatin pro-region expression system in non-endocrine cells" as providing evidence of a reasonable expectation of success. This statement is made despite the fact that both references teach that pro-region expression systems resulted in (i) low levels of somatostatin secretion and (ii) inefficient processing of pro-somatostatin.

The present Office Action dismisses these teachings away as unpersuasive, and appears to credit one sentence in Warren as being dispositive on the issue of whether Warren and Patel teach away from the present invention. In support of the rejection, the Office quotes the following statement from Warren (at page 553) emphasis added:

> However, in the absence of quantitative data we cannot accurately compare either the levels of proteolytic processing or secretion of the two hormones in these cell types.

Applicants respectfully submit that this statement does not contradict the numerous other statements in Warren and Patel that teach away from the present invention. For example, the above-quoted statement is immediately preceded by the unequivocal statement that the "data show a relatively low level of [processed somatostatin] SRIF secreted compared to insulin secretion in AtT-20 cells." The sentence quoted by the Examiner merely states that without quantitative data, "we cannot accurately compare" secretion and processing efficiency of insulin in AtT-20 cells and preprosomatostatin in COS cells. This inability to accurately compare secretion and processing efficiency of two different proteins in two different cell types, does not contradict the unambiguous, if non-quantitative, observation in Warren that very low levels of processed somatostatin were detected in non-endocrine cells.

A skilled practitioner, considering the teachings in Warren as a whole, would understand that lower levels of processed somatostatin were detected in non-endocrine cells, relative to what would have been observed in endocrine cells. For example, Warren states that their results strongly suggest that propeptide proteases are present in COS cells, "albeit at a rather low level. In this context it is noteworthy that we did not observe cleavage of proSIRF to SS-28 ... this suggests that the COS cell processing does not reflect the true physiological processing of preproSIRF." (See page 553, emphasis added). Warren also engages in a lengthy discussion that

describes the authors' (apparently surprising) ability to detect processed somatostatin using HPLC. This ability was noteworthy because others had previously failed to detect secreted mature hormone peptides in non-endocrine cells using radiographic methods (see, e.g., Warren at page 553, paragraph bridging left- and right-hand columns). This discussion clearly implies that in non-endocrine cells, processed propeptides were expressed at levels that were too low to be detected radiographically, whereas in endocrine cells, processed propeptides were detectable by SDS-PAGE and radiography.

Applicants respectfully submit that Warren's inability to quantitatively compare expression and processing of insulin in AtT-20 cells and preprosomatostatin in COS cells does not, in any way, undermine the report in Patel that preprosomatostin was secreted and processed much less efficiently in non-endocrine cells than in endocrine cells (see Patel at pages 31-32 and Figure 2). Patel not only confirms Warren's non-quantitative observations that expression of processed somatostatin in non-endocrine cells is poorer than in endocrine cells, Figure 2 in Patel supplies some of the quantitative data that Warren lacked, i.e., data showing that somatostatin was processed much less efficiently in COS (non-endocrine) cells relative to AtT-20 (endocrine cells) cells. Thus, Patel supplements the state of the art as it was when Warren was published.

Given the many passages and results in Warren and in Patel that teach away from the effectiveness of processed somatostatin expression in non-endocrine cells, Applicants fail to see why the Office has selected for special focus this one sentence from Warren. As a whole the references disclose very poor expression of processed somatostatin in non-endocrine cells, and thus they teach away from using non-endocrine cells for the expression of constructs expressing peptides linked to a somatostatin pre-pro region.

Finally, contrary to what is stated in the Office Action, applicants have not argued that Warren's age discredits Warren as a prior art reference. Rather, Applicants have pointed out that Warren and Patel are not inconsistent. In fact, some of the data Warren complains was missing and that could have been used to "more accurately" compare the (in)efficiency of somatostatin expression in non-endocrine cells and endocrine cells, has

been provided by Patel. Thus, the Office Action's citation to *In re Wright* (see the Office Action at page 7) is not appropriate here.

For the reasons above, applicants respectfully request that the rejection of claims 14, 17, 19, 21-35, 37-46, 83-89, 92 and 93 be reconsidered and withdrawn.

Claim 36 has been rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of Sevarino, Stoller, and Warren, as applied to claims 14, 17, 19, 21-35, 37-46, and 83-89, and further in view of U.S. Patent 6,010,883 (Nagai). Applicants respectfully traverse this rejection.

The Office Action alleges that Sevarino, Stoller, and Warren render obvious a cell transfected with a nucleic acid encoding a fusion protein comprising a signal peptide, a pro-region of a somatostatin, a cleavage site, and a desired protein. Nagai teaches the use of blood coagulation factor Xa cleavage sites in recombinant fusion proteins, including recombinant fusion peptide hormones.

Applicants respectfully submit, for the reasons discussed above, that Sevarino, Stoller, and Warren, singly or in combination, do not render obvious a non-endocrine cell comprising a nucleic acid sequence encoding a fusion protein that comprises a signal peptide, a pro-region of a somatostatin, a small non-somatostatin peptide, and a blood coagulation factor cleavage site. In fact, these references teach away from a cell with the currently claimed limitations. Nagai does not remedy the deficiencies of Sevarino, Stoller, and Warren. Although Nagai does teach the use of blood coagulation factor Xa cleavage sites in recombinant fusion proteins, Nagai clearly provides neither (i) a motivation for combining Nagai, Sevarino, Stoller, and Warren nor (ii) the requisite expectation for successfully making and using the non- endocrine cell recited in claim 36. Therefore, Sevarino, Stoller, Warren and Nagai, individually or in combination, do not render obvious the cell recited in claim 36.

For the reasons above, applicants respectfully request that the rejection of claim 36 be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that all claims are in condition for allowance, which action is requested. Enclosed is a $490 check for the Petition for Extension of Time fee for a three-month extension. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 10278-014001.

Respectfully submitted,

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